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SOURCEBOOK UPDATE: INTESTINAL SMOOTH MUSCLE CONTRACTILITY AND AUTONOMIC CONTROL

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ABBREVIATED TITLE

Introducing authentic inquiry into a laboratory practical

KEYWORDS

Mammalian gastrointestinal physiology

Autonomic receptor pharmacology

Laboratory practical

Authentic inquiry

Problem-solving

ABSTRACT

This laboratory practical requires first year students to anticipate the effects of drugs active at cholinergic and adrenergic receptors on gut motility in order to design experiments during an authentic inquiry exercise. Rather than specifying a strict sequence of drug additions that aim to provide ideal demonstrations of pharmacological and physiological antagonism, I have instead designed switches into the drugs provided and set students, working in small teams, the task of identifying the switched drugs – an inquiry activity. To extend the teamwork aspect, laboratory reports were submitted by the student teams rather than individual students. Staff observed that discussions within the teams was stimulated by the inquiry-led nature of the practical. The quality of the laboratory reports submitted by teams were substantially improved over the individual reports submitted in previous years (*students previously worked in teams but simply followed a list of prescribed experiments and wrote individual reports*). Although, in conversation, teams of students had an improved understanding of the regulation of gut motility by the parasympathetic and sympathetic divisions of the autonomic nervous system and could readily distinguish between pharmacological and functional antagonism, no attempt was made to evaluate learning because the revision was triggered the observed effect of a technical error and was not otherwise planned. It is likely that laboratory practicals, in general, would benefit from inclusion of inquiry.

INTRODUCTION

Objectives and Overview

This sourcebook update article describes a laboratory practical that relies upon the same underpinning knowledge of gut muscle contractions, the enteric nervous system, the influence upon contractions of the parasympathetic and sympathetic branches of the autonomic nervous system, and utilises the same experimental approach as previously described (21). Whereas the focus of the activity described by Montgomery *et al*. (2016) was a formal investigation of the properties of agonist and antagonist drugs and the principle of functional antagonism, the activity I describe was designed to incorporate elements of authentic inquiry, exposing first year students to scientific method by the inclusion of iterative observation,

hypothesis formulation, experimental design, and the interpretation of data (3). Students in teams of four are provided with a panel of eight drugs, complete with information about each drug (receptor specificity, agonist or antagonist, molecular mass, *etc*). Students are briefed that two of their eight drugs have been switched and that their task is determine, by reference to the effects of the drugs on spontaneous contractions of mammalian gut, which two drugs are switched. The value of discussion and teamwork is further strengthened by the requirement that teams submit jointly authored laboratory report (25).

Background

The key background is provided in an earlier sourcebook (21). Whilst it is clearly important to demonstrate the properties of receptors and phenomena such as competitive antagonism, if '*physiology is the study of how cells, tissues and organs function*', as suggested by the American Physiological Society, there can be few tasks more important for the novice student than to understand the autonomic nervous system. The autonomic nervous system is delicately complex and defies a simple or trivial explanation and yet it is often taught in a simplistic, fact-dominated fashion (17). Moreover, first year laboratory practicals tend initially to have simple aims, focusing often on incremental development of basic laboratory skills, such as pipetting, adequate recording of experiment data, taking measurements and making judgements on the best way to visualise and describe data (10). Another, less often articulated aim, is to increase students' awareness of variation in the responses of biological tissue and the implications this has for experimental design and the interpretation of experimental results. The above argument notwithstanding, laboratory practicals that provide opportunities for students to learn how receptors for the main autonomic neurotransmitters influence the contraction of the gut are increasing difficult to defend, both ethically and economically and are increasingly replaced by software simulations (22). However sophisticated, the software versions ultimately lack true authenticity and student engagement with them can be superficial and perhaps diminished for precisely this reason (12). To address these concerns, I devised a gut organ-bath experiment that presents teams of first year students with an intellectually challenging problem that can be solved by the application of the scientific method (3, 8, 10, 11, 18). In doing so, I turned a recipe-driven practical into an inquiry-led, team-based activity providing the opportunity for students to learn far more than just the regulation of gut motility.

Students studying pharmacology and physiology require a practical understanding of the nature of receptor agonists and antagonists and to appreciate that stimulation of a receptor can inhibit as well as stimulate a physiological variable, such as gut motility. I elected to move much of this cognitive effort out of the laboratory practical by providing information about each drug in advance of the practical. The student's incentive to engage with this material is a mandatory on-line quiz scheduled prior to the laboratory session (18). This effort was rewarded within the laboratory session because it facilitated completion of a table of expected results (described later) that served to highlight results that were unexpected and, therefore, suspicious, warranting replication and close scrutiny.

In order that the nature of the switch did not become widely known by word of mouth between cohorts, two switches were developed. In **Switch One** the two drugs that are switched are propranolol, an antagonist at beta adrenoceptors and phentolamine, an antagonist at alpha adrenoceptors. In **Switch Two** the two drugs that are switched are atropine and noradrenaline**.** Both drugs in Switch Two will depress motility.

By redesigning the experiment to include the elements of inquiry my aim was to transform a recipe-driven exercise into an active learning experience that would increase engagement and augment understanding of receptor pharmacology and experimental design. Finally, by requiring a team laboratory report, rather than individual reports, I aimed to foster the attitudes necessary for effective and collaborative teamwork (14) with the added benefit of reducing the marking load by a factor of four (7).

Learning Objectives

After completing this activity, the student will be able to:

1. CONTENT KNOWLEDGE: Define key terms used in explaining autonomic nervous system, receptor agonist, receptor antagonist, competitive antagonism, functional antagonism.
2. CONTENT KNOWLEDGE: Calculate the molar concentration of a stock solution from the mass of the substance, the volume of the solution and formula mass.
3. CONTENT KNOWLEDGE: Describe and explain the consequences of exposing a section of mammalian ileum to a variety of receptor agonists and antagonists.
4. CONTENT KNOWLEDGE: Complete a table of expected results based on information provided on the properties of drugs and the pharmacological regulation of gut motility.

5. PROCESS SKILLS: Handle drug solutions in a safe and controlled manner. Accurately dispense drugs using the appropriate pipette.
6. PROCESS SKILLS: Perform experiments with careful planning, accurate observation and recording of results
7. PROCESS SKILLS: Recognise findings that do not correspond to those in the table of expected results
8. PROCESS SKILLS: Design experiments to i) generate a hypothesis regarding the drug(s) that do not result in the action expected, and ii) test the hypothesis framed in i).
9. PROCESS SKILLS: Explain experiments listed in 8 and request sample of 'known' drug in order to validate their findings.
10. PROCESS SKILLS: Collaborate as team to complete an experimental report.

Activity Level

This activity is used as a learning opportunity for students in their first year of undergraduate study in physiological sciences and veterinary science programme but would also be suitable for other biomedical science or healthcare professional programmes such as medicine.

Prerequisite Student Knowledge or Skills

Before undertaking this activity, students should have a basic understanding of:

- Smooth muscle excitation-contraction coupling
- The receptor theory of pharmacology
- The distinction between receptor agonists and receptor antagonists
- The principle of competition at receptors and the properties of antagonism that is competitive
- The division of the autonomic nervous system into the sympathetic and parasympathetic nervous systems
- The transmitter substances released by the post-ganglionic fibres of sympathetic and parasympathetic nerves, that most clearly influence motility.

Clearly the required laboratory skills can be varied to take account of the level and expertise of the students as well as the aim of the laboratory exercise. The level of support and instruction described here is appropriate for first year students who are as yet unfamiliar with handling biological tissue. The justification for providing students with working organ bath preparations is that it reduces the cognitive load (27-29) and enables the students to focus fully on the principle task, that of planning of their experiments. On that basis, students should know how to:

- Observe safe laboratory practices
- Perform basic calculations to establish the final concentration of the key drugs used
- Keep adequate records of experiments performed
- Collect data carefully and accurately

Time and Resources Required

The activity described was designed to be completed by first year students within a single three hour laboratory practical. Prior to arrival, it is expected that students complete their pre-reading of the laboratory notes, which explain the concepts, receptor pharmacology and the influence of the divisions of the autonomic nervous system on the gut (to aid in achieving content learning objectives 1, 2 and 3), and complete an online pre-practical quiz (5, 13).

The laboratory practical is run twice to accommodate approximately 200 students working in teams of 4 (25 sets of experimental equipment). In terms of staff resource, the practical is led by an experienced teacher, ideally the teacher who delivered the lectures on GI physiology or the autonomic nervous system, with the support of several teaching assistants. The teaching assistants are either postgraduate students (research assistants) who contribute some of their time as teaching assistants, or else medical demonstrators, who are typically medically qualified and are employed specifically to help deliver laboratory teaching. Teaching assistants and medical demonstrators (hereafter collectively referred to as teaching assistants) are required to be familiar with the underlying physiology and pharmacology and are provided with training specific to each laboratory practical. This training is scheduled in the week before the laboratory practical runs. We have found that intensive laboratory exercises of this sort require one member of teaching staff for every six

teams and so the exercise requires four staff. Additionally there is need of expert technical support and we would typically have the support of three laboratory technicians. Laboratory-based inquiry exercises of this sort could be easily adapted to provide a longer-term experimental challenge in which students, perhaps in smaller numbers, investigate a problem over several weeks. Such an approach could be adopted effectively if several techniques were required, perhaps starting with exploratory organ bath experiments to establish a target, followed by focussed experiments of the student's design, perhaps to define drug efficacy and potency, before using radio-ligand binding approaches to characterise the receptor properties (6). The scope and design of such experiments would need to carefully consider the aims in terms of laboratory skills (*e.g.* handling tissues), experimental design, data presentation, statistical analysis and report writing.

METHOD

The methods and materials required to set up the gut bath and record the contractile activity are explained in detail in a previous sourcebook report (21). Below, I provide only the information specific to the laboratory exercise in focus. The following equipment and supplies are needed:

Equipment

1. Organ baths and necessary recording equipment
2. Measuring cylinder (10 or 25 ml maximum volume – choice dependent upon bath volume) used to charge the organ chamber with a known and standardised volume of physiological salt solution after adequate washes are completed.
3. 1 ml syringes for application of drugs (0.1ml is the volume typically used – resulting in x100 dilution). The experiments do not require the application of precise concentrations of the drugs and so the accuracy of volumetric pipettes is not required.
4. 600 ml glass beaker labelled '**for collection of fresh physiological salt solution'**
5. 600 ml glass beaker filled with distilled water, labelled '**distilled water for rinsing syringes'**
6. 600 ml glass beaker labelled '**waste water from syringes'**
7. Copy of the **pre-practical support materials** document (Appendix 2; https://goo.gl/qnZ9bR).
8. Copy of the **neuromuscular practical protocol and proforma** (Appendix 3; https://goo.gl/r9B663).

Solutions

1. Reservoirs of pre-gassed physiological salt solution that teams can draw on as required.
2. Drugs (5 ml of each per team) contained in bijou bottles and placed in drilled wooden blocks that students are instructed to keep tidy as part of good laboratory practice: Acetylcholine ($5.5x10^{-7}$ M and $2.75x10^{-6}$ M), Atropine ($2.88x10^{-7}$ M), Noradrenaline ($6.26x10^{-7}$ M and $6.26x10^{-6}$ M), Adrenaline ($9.1x10^{-7}$ M and $9.1x10^{-6}$ M), phenylephrine ($1x10^{-6}$ M), isoprenaline ($8.07x10^{-7}$ M), phentolamine ($1.01x10^{-5}$ M), propranolol ($8.12x10^{-6}$ M). Note that stock solutions of both adrenaline and noradrenaline contain 1 $mg.ml^{-1}$ ascorbic acid as an antioxidant and that fresh acetylcholine is purchased just prior to the experiments.

Animal Subjects

The ileum is typically isolated from guinea pig, although rabbit works well also. No specific ethical approval or personal licence is required if the animals are euthanized according to Schedule One of 'The Use of Animals in Scientific Procedures' Act (1986) (21).

Instructions

Preparation prior to the practical

In advance of data gathering, students, who work in teams of three or four, must calculate the concentrations of each of the stock solutions of drugs they will use from the information provided (molecular mass, mass per millilitre), (ii) complete a table of likely effects (anticipated results) and (iii) observe a demonstration of how to use the available equipment, document the progress of the experiments and decide what measurements are necessary and how to make those measurements.

In our programmes this practical is scheduled in the first academic term and is the first laboratory session in which there is a significant degree of inquiry, an element previously shown to aid engagement and learning (2, 15).

(1) Calculating concentrations of drugs used

The table below gives the composition of the stock drug solutions in terms of micrograms per millilitre and provides also the molecular mass of each drug. Students are encouraged to become familiar with different expressions of concentration (mmoles per litre, %weight by volume and grams per litre, *etc*) and so completing this table provides an opportunity to practice the necessary conversions. Students are encouraged to validate their calculations using both estimation and back-calculation.

Table 1: Drug concentration calculations

(3) Table of likely effects

The experimental protocol document (appendix 3; https://goo.gl/r9B663) provides all the information necessary for students to anticipate the likely effect of each receptor agonist. By extension, with some assumptions regarding relative concentrations, students can anticipate the effects of agonists added after pre-addition of specific receptor antagonists. Students are thus required to devise a table of likely effects (anticipated results). The experimental protocol offers the following guidance, '*The team will use the information in the Introduction and the material provided in the class to construct a table of likely effects of adding the various agonists and antagonists (refer to introduction and lecture notes). This should initially include addition of agonists alone and then in the presence of appropriate antagonists. This table should be attached to your practical notebook – do not discard!'* (text from point ii in **ORGANISATION**, Appendix 3).

Table 2: Partially completed table of likely effects

(4) Demonstration of ideal experimental approach

As this laboratory practical is the first one that makes use of the organ bath, one person from each team is nominated to observe one of several parallel demonstrations of the experimental equipment, each run by an experienced academic teacher or trained teaching assistant. It is the nominated person's responsibility

to take notes of the procedure, ask questions for clarification and be prepared to explain the points covered to the other members of their team.

Conduct of the experiments

Set up

By the start of the experiment, all the experimental stations (n=25) have been setup by the teaching assistants. With smaller numbers of students and setups, the students could be required to set up their own preparations but, given our cohort size and mindful that the students' main aim is to design a series of experiments to solve a logical problem, the additional tasks would only add to the cognitive load (28) and increase the likelihood that teams of students would fail to complete the experiments in the time available, learn little and become disillusioned. The equipment and setup procedure has been previously explained in detail (21). A maximum of four students operate each station and the laboratory is run twice (morning and afternoon) in order to accommodate the present cohort (n=200). Although there is merit in having students learn to handle biological tissue, these are first year students and it is the first time they have used the mammalian gut preparation or the equipment. In addition, there are a great many stations for teaching assistants (n = 4) to supervise and advise.

Plenary session

The laboratory session begins with a plenary by the lead teacher that serves to:

1. Remind teams need to demonstrate good laboratory practice – wearing lab coats and gloves, washing hands at the end of the experiment, to refrain from eating and drinking (or chewing of pencils, *etc*);
2. Remind teams that the aim is to investigate the effects on contractile activity of a number of substances (some endogenous and some man-made);
3. Remind everyone to consult a document, present at each station, listing drug names, molecular masses and pharmacological characteristics. This document is also available to students on-line (Appendix 2 **pre-practical support materials** - https://goo.gl/qnZ9bR);

4. Remind everyone that there has been a deliberate mistake made in labelling the drugs, provided at each experimental station, specifically that two of the drugs have been switched and the objective of the laboratory session is to discover which two are switched. It is explained that any investigation will require teams to develop some expectation of what each drug will do; either to the activity directly, or to the action of other drugs *i.e.* an antagonist drug will inhibit or block the action of an agonist drug;
5. Highlight the need for students to communicate effectively and work as a team – there is a lot to do;
6. Explain that one member of each team is responsible for ensuring that the recording device is working and that they know how to make the observations e.g. recording of control activity, adding drugs, period of observation, double rinsing and marking the record adequately to allow off-line measurements, *etc*. The required approach is explained and demonstrated by staff after the plenary session;
7. Explain that whilst their team-mate attends the demonstration, the remaining team members should to re-read the experimental protocol and the pre-practical support materials and construct a table of likely effects;
8. Remind teams that the drugs are labelled with the team number and not to mix up the bottles with those from other teams. Neither should they mix the tops of bottles as this will contaminate the contents of both bottles;
9. Encourage teams to note their team number and the colour (green or yellow) of the wooden blocks in which the drug bottles sit. Both these pieces of information will be required to complete the experimental report;
10. Make clear that there are multiple switches and so not to expect to record findings similar to the teams nearby;Stress that teams will need ultimately to confirm their suspicions about which drugs are switched. To this end ***standard*** drugs are available from teaching assistants but teams must ask for each drug by name *i.e.* 'I need some real adrenaline please.' Access to reliable standard drugs will enable teams to confirm their conclusions or send them back to the drawing board. Teams

should be warned that they will need to explain to the demonstrator what experiments lead them to suspect that a given drug is the subject of a switch. This will involve reference to their table of likely effects and recordings of contractile activity that do not conform to the list of likely effects, along with sound reasoning as to the experiment(s) that would distinguish between the possible explanations for the results obtained;

11. Remind teams that the laboratory report should be submitted one week later. It should clearly state which switch (yellow or green) they were working on and their team number;
12. Remind teams that a document (pre-practical support notes) is available online for revision purposes (https://goo.gl/qnZ9bR).

Discretionary points

The points below are typically not disclosed to students but could be used if time constraints are otherwise an issue.

- **Switch One** – Green. **(Propanolol switched with Phentolamine)**
  At the discretion of staff running the laboratory, it is possible to give a clue; that both of their switched drugs work on sympathetic receptors OR that they are both antagonists (don't define sympathetic in this case). The latter is the bigger hint.
- **Switch Two** – Yellow. **(Atropine switched with Noradrenaline**)
  At the discretion of staff running the laboratory, it is possible to give a clue; that one of their switched drugs works on parasympathetic receptors and the other on sympathetic receptors.

Demonstration of how to make recordings

During the demonstrations of the equipment, the representatives of each team are advised strongly to consider several factors to ensure that their records are easy to visualise which aids interpretation and incorporation into the laboratory report:

1. Choose a chart/screen speed that enables changes in amplitude to be seen clearly. On a computer screen, this equates to a screen width of about 10 to 15 minutes.

2. Check and if necessary reset the minimum force to be not less than 25 mN.
3. Periodically monitor the minimum force or maximum length and readjust as required.
4. Monitor and adjust bubbling. Bubbling maintains the correct pH, but also mixes the drugs.
5. Plan each experiment before adding the first drug and ensure all drugs required are close at-hand.
6. Ensure that there is a minimum of one minute of control activity recorded before adding the first drug. Consider the duration required if the force is cycling over time (e.g. minute rhythm).
7. Take care to label (annotate) each event, including washes and adjustments to resting force.
8. For simple experiments – those involving only a single agonist – the drug should be applied for only as long as required to observe a clear effect. It is **NOT** necessary to wait until the effect is fully developed and stable. As soon as the effect is clear, drain the chamber and flush twice (Figure 1).
9. The level of contractile activity (motility) should alter with the application of any drug that acts as an agonist. If there is no response within 10 seconds, the concentration should be increased.
10. Acetylcholine, noradrenaline and adrenaline are supplied in two concentrations (1 and 2). If there is no response to 0.3 ml of solution 1 then instruct students to drain and flush the chamber twice. Wait for the activity to return to a stable level, typically two minutes, and then add 0.1 ml of solution 2. A further 0.2 ml of solution 2 can be applied if there is no response to the first addition. If, after a total of 0.3 ml of solution 2, there is no response then seek the help of a teaching assistant.
11. Typically, antagonist drugs should be added after the agonist so that the effect of the antagonist can be readily distinguished.
12. Description of drug effect requires consideration as it is possible to describe changes in motility in a variety of ways. Firstly, amplitude (difference between maximum and minimum force). Second, absolute level of force. Finally, average force (typically the mid-point between the maximum and minimum force (see figure 1)). Teams will need to make a judgement as to what description communicates most clearly the nature of the effects they observe.

Figure 1 – Example of an experiment to demonstrate the ideal duration of the exposure to drugs.

Common Errors

A common error is exposing the preparation to drugs for longer than is necessary. This extends the period of time required for the effects of drugs to reverse and for motility return to a stable level. Another error is incorrect or incomplete labelling of the experimental record. These procedural errors can result in slow progress which frustrates the students, and losing track of the experiments which frustrates everyone. Spotting these errors early is important as they can lead to the loss of a learning opportunity and the waste of the experimental animal. The ethical implications of this should not be ignored. Trained teaching assistants should be on hand to spot errors early and help students recognise and rectify them as soon as possible.

Safety Considerations

The reader is directed to the earlier sourcebook report (21).

RESULTS

The laboratory practical described here replaced a recipe-driven practical in which students observed changes in gut motility in response to the addition of one or more drugs. Given the relatively low bar of grasping the effects of parasympathetic and sympathetic systems on motility and the capacity to follow a numbered list of instructions, engagement was poor and learning was insubstantial.

With the introduction of inquiry came a wholesale increase in engagement and learning. To succeed in the pre-laboratory on-line tests, students must demonstrate understanding of the key receptors, as well as the characteristic properties of competitive antagonism. They have also to begin to recognise the distinction between pharmacological and physiological antagonism. Thus, the inclusion of an inquiry element requires students to operate higher up Bloom's taxonomy; at analysis and synthesis, something that has been shown to increase engagement (16).

One of the key tasks that students complete is a table of expected results. Completion of this table appeared often to be predictive of good progress by teams as it provided a clear framework that was visible to all team members and to teaching assistants, against which experimental findings might be compared. A complete and comprehensive table is shown in table 3.

Table 3 – complete table of expected results

Expected Results

Switch One (Green)

The two drugs that are switched are **propranolol**, an antagonist at beta adrenoceptors and **phentolamine**, an antagonist at alpha adrenoceptors. Agonists of these receptors would be expected to reduce motility. Consultation of table 3 identifies the following diagnostic results and critical experiments:

a. The drug labelled as propanolol will very effectively inhibit the actions of noradrenaline (predominantly an alpha agonist) which it should not do (table 3, experiment 11).

b. The drug labelled as propanolol will antagonise the effects of the alpha receptor selective agonist, phenylephrine. Again, this should not happen (table 3, experiment 17).

c. The drug labelled as phentolamine will partially inhibit the effects of adrenaline but this is to be expected however it will also inhibit (powerfully) the action of the beta-receptor-selective agonist, isoprenaline. This would be unexpected (table 3, experiment 19).

Teaching assistants are briefed to discuss observations with the teams and to advise them that they can safely assume the switch is not adrenaline with noradrenaline on the basis that the experiments they can easily perform would not satisfactorily separate the effects on motility of these non-selective agonists. The students will be able to deduce that the switch involves drugs that are active at adrenoceptors, but they will be unable to discriminate between the two options for the switch, (1) the agonists, phenylephrine and isoprenaline, or (2) the antagonists, phentolamine and propranolol. The critical experiment will require that the students request a sample of standard phentolamine. This will very effectively inhibit the effect of what they have labelled as phenylephrine, which is expected as the alpha agonist, phenylephrine, is not subject to the switch. By extension, the standard phentolamine will have no effect on the drug they have labelled as isoprenaline. As a final confirmation, students may wish to request the standard isoprenaline, a beta-

receptor agonist, which will be very effectively inhibited by what they have labelled as phentolamine, an alpha receptor antagonist. Some teams may also elect to combine the standard phentolamine with the drug they have labelled as phentolamine which together will inhibit both phenylephrine and isoprenaline as well as noradrenaline, a finding only possible if the drug labelled as phentolamine contains instead, propranolol.

Switch Two (Yellow)

The two drugs that are switched are **atropine** and **noradrenaline.** Both will depress activity. Diagnostic results and critical experiments:

a. The drug labelled as noradrenaline will depress activity (table 3, experiment 4), as expected, but its actions will not be sensitive to either phentolamine (experiment 10) or propranolol (experiment 11), inhibitors of alpha and beta receptors, respectively. The ineffectiveness of phentolamine and propranolol does need to be confirmed by their simultaneous addition (experiment 12) which is something that teams are not explicitly instructed to test, and the effectiveness will not conform to the table of likely effects.

b. The action of the drug labelled as noradrenaline will inhibit the action of acetylcholine (table 3, experiment 20), even in the presence of both phentolamine and propranolol (alpha and beta adrenoceptor antagonists). This would be an unexpected observation.

c. An experiment that may not initially recommend itself is to apply phentolamine or propranolol (or both) prior to the drug labelled as atropine (not listed in table 3 as it would not be a rational experiment based on expected results in the absence of a switch). The action of the drug labelled as atropine will be blocked by phentolamine and will leave the effect of acetylcholine intact. This would be an unexpected observation.

Switch two is particularly useful as it can be used to explore two important concepts. Firstly, the notion of a resting, or unstimulated level of activity *in vitro*. As explained by Montgomery *et al.* (2016), and the pre-practical support materials (and the accompanying lecture course), there is an ongoing level of activity within the enteric nervous system and perhaps also the post-ganglionic fibres of the parasympathetic system. Both

of these would tend to augment levels of motility. The application of atropine would be expected, therefore, to depress this tonic stimulatory influence and so result in an overall inhibition of motility. Secondly, the effects are a demonstration of two types of antagonism – pharmacological antagonism and functional antagonism. Acetylcholine, acting via muscarinic receptors, increases motility. This effect is inhibited by real atropine which blocks the muscarinic receptors. This is pharmacological antagonism. The stimulation induced by acetylcholine can also be inhibited by agonists that stimulate either alpha- or beta-adrenoceptors, as stimulation of these receptors powerfully inhibits motility. The antagonism by noradrenaline of the effect of acetylcholine is termed functional antagonism. Thus, the reduction of activity observed when the drug labelled as atropine is applied is an example of functional, rather than pharmacological antagonism.

CONCLUSIONS

Inquiry Applications

This laboratory practical is ideal for basic science students (physiology and pharmacology) as well as veterinary and medical students. Typically, laboratory practicals in first year undergraduate physiology incorporate limited inquiry, but formulated as described, this laboratory practical equates most closely to Hegarty's level 2A inquiry activity (9). Hegarty's levels range from zero to three with a subdivision of level two into A and B. Level zero laboratory practicals are quite common in first year teaching and are characterised by close prescription of all elements, including aim, equipment, method and answers (outcomes). At level three nothing is specified and the students must identify their aim(s), their experimental approach, including the equipment they require, the experimental design and decide in advance how they will describe their data and use it to address their initial aim. Although the classification of Hegarty includes **method** and **answer** as variables in the schema, it neglects to include experimental design. The aim, materials and method can all be specified and fixed, but organ bath experiments that involve eight drugs, from which two are switched, has a high degree of freedom and a significant intellectual challenge exists if the teams are to design meaningful experiments. No script is supplied, save that teams should apply the agonists first and only then explore the action of the agonists in combination with antagonists. This approach does mean the

448 experiments differ between teams and so results cannot easily be pooled and instead a great deal of
449 responsibility falls to the teaching assistants who periodically engage each team in discussion. This too is a
450 strength as it obliges the students to describe their experiments concisely, articulating their reasoning in
451 selecting plausible candidates for the switch and explaining their rationale for testing their hypotheses and
452 using further confirmatory experiments or else the standard drugs (4).
453 Prior to university, students have limited experience of designing experiments and so their skills are typically
454 poorly developed. The approach required to successfully complete this laboratory practical are fundamental
455 to a research-based education (15, 19, 24). The laboratory practical has also been designed to encourage
456 students to think in a more holistic fashion about both pharmacology and physiology, helping to cement key
457 concepts of receptor pharmacology and raising awareness of variation in responses of living tissue and the
458 threshold concept (20) of uncertainty (23). Introducing authentic inquiry into laboratory practicals is not easy
459 as both staff and students are exposed to the risk of failure and staff may initially lack the relevant experience
460 necessary to manage the students' expectations and the anxiety they display when required to plan their
461 own experiments (26). Accordingly, the role of the lead teacher and the teaching assistants is of key
462 importance. Careful and close observation of progress and reasoning are key to ensuring that students do
463 not become confused by results that are less than ideal. To this end, the School invests time and effort to
464 ensure that everyone involved in our laboratory practicals receive guidance and training about one week
465 prior to the practical sessions. To facilitate this we provide staff versions of the practicals in which there are
466 notes designed help ensure that explanations are consistent with the content of lectures and between
467 teaching assistants. As our post-graduates have often read for undergraduate degrees in other universities,
468 and so will have different experiences in terms of lecture content and laboratory exercises, we regard this
469 programme of training and orientation to be necessary for the undergraduate students' experience of their
470 laboratory practicals. Instilling in undergraduate students the principles of good experimental practice is
471 important in this regard because if the experiments are not carefully conducted and adequately documented,
472 there is a high probability that effort will be made to understand the results of mislabelled experiments. The
473 principle could be introduced that experiments yielding unexpected results should immediately be repeated
474 and accepted for consideration only if the initial finding is replicated (11).

The Laboratory report

Each student team is required to submit a short laboratory report in which they summarise their findings in a table and show traces only for experiments producing unexpected results. Students are instructed that reports should include experiments designed to test their hypotheses regarding the switched drugs and to explain the rationale for these experiments. The laboratory reports have a prescribed format that specifies the required sections as well as a maximum overall length. The sections are:

- **Coversheet** – identifies the colour of their experiment (yellow or green) and includes a declaration of academic integrity that is signed by each team member;
- **Summary statement** – this is typically a simple statement identifying which drugs the team had identified as subject to a switch;
- **Introduction** – students are directed to provide an outline that would enable a reader to understand the aim of their investigation. The instructions students receive remind them of the overall length of the report. It is expected that teams include a brief summary of the essential nature of drug action in order to define agonist and antagonist drug actions. It is expected that the report makes it clear that all the antagonists available to them were competitive antagonists as this was highlighted in the supporting material provided to students in advance of the laboratory practical. It is also expected that the students include an overview of the effects of key receptors on the motility of mammalian ileum. The use of diagrams from their lecture courses is explicitly permitted but an attribution to the lecturer and lecture is expected. Students are instructed to either start or conclude the introduction with a statement that explicitly lists the aim(s) of the experiments reported. Most teams write something equivalent to '*the aim of this investigation is to identify two mislabelled drugs that have been switched, out of the eight drugs provided, by studying their action on a section of rabbit ileum'*. Some teams chose to include a preliminary table of expectation in the introduction, others included this in the method.
- **Method** – students are instructed NOT to repeat the method provided in the experimental protocol document (appendix 3). Students were asked to include only information that was additional to the

standard instruction and would be useful to the reader. So, for example, some groups stated that when studying the effects of antagonist drugs, they added the agonist first, followed by the antagonist, typically with a justification such as '*in order to see more clearly the effect of the antagonist drug*'.

- **Results** – Most teams summarise their results into a table based on the table of expected results that they are directed to complete during the laboratory practical. For the green switch (phentolamine and propranolol) this is typically formatted as shown in table 2.
  The same or similar tables are often used to highlight the findings that do not match expectations and annotated traces of the tension recordings are typically included. The best groups make explicit links to their hypotheses and these unexpected results and then to subsequent experiments that they conducted in order to further investigate the unexpected findings. For the best marks, the report will have included all the logical tests listed in the **expected results** section above before describing the effects of the standard drugs that they requested to test their tentative hypothesis for the switched drugs.
- **Discussion** – In this section the report steps through only the findings they believe to be relevant to the task set them; that if identifying the switched drugs. The high marks are reserved for reports that follow an appropriate cycle of planning, followed by observation and then reflection upon the original plan in order to judge the meaning of the observation. For example, one team wrote, '*by inspection, the first disagreement between the predicted and observed response occurred on the addition of phentolamine to noradrenaline in that the action of noradrenaline was not antagonised as we had expected. This was confirmed by adding phentolamine after phenylephrine (the team included a figure of this), where again the expected result was not observed and there was no recovery of contraction even when the concentration of phentolamine was increased ten-fold. Combined, these results suggest that phentolamine has been mislabelled*'. This team went on to explain how their findings for propranolol was similarly suspect, along with the several additional experiments which they felt, with some justification, identified propranolol as a likely switch. The

best marks were given to reports that laid out their report in a logical order, assuming their findings were accurate and were concise.

Evaluation of Student Work

The aim of the inquiry students undertake in this laboratory practical is to identify which pair of drugs are subjects of a switch. In the initial years, most of the teams did not include details of all the experiments that would provide compelling evidence for the switch. Instead, most teams were seemingly content to go immediately from a suspicion to a test using the standard drug. For example, teams asserting that their atropine was switched with noradrenaline typically elect to show only the effects of standard atropine. It would make a stronger demonstration to first test what they have labelled as atropine, but suspect to be noradrenaline, in the combined presence of phentolamine and propranolol. Their hypothesised switch (atropine for noradrenaline) implies that both phentolamine and propranolol are as they appear and should completely inhibit the effects of the drug labelled as atropine (in reality, noradrenaline). In most cases these logical checks were not included in written reports although the experiments were often done, based on testimony of the teaching assistants. This might reflect the students' experience in secondary education which celebrates correct answers and depreciates the value of the intermediate elements of observation and reasoning (1). The solution, arrived at over several years, had two elements. First, staff were made aware that students were failing to build a compelling case for a switch before requesting a standard drug. This realisation led to a change in the training to ensure that staff (lead teacher, teaching assistants and technical staff) required teams to explain the experiments they had done and how their findings led them to conclude which drugs were switched. If logical tests, such as those listed above, were not complete, teams were nudged in the right direction but not told which experiments they should perform.

The distribution of marks for the exercise has also evolved over time. Currently, the mark scheme includes the following elements:

Element	**Grading categories**	**Marks**
Overall finding	Good/Adequate/Poor	15%

Evidence of logical observation and experimental design (informed by illustrations and report)	Good/Adequate/Poor	30%
Inclusion of illustrations that support interpretation	Good/Adequate/Poor	25%
Report (document format, clarity and accuracy of expression, spelling, grammar)	Good/Adequate/Poor	30%

Students have sight of this scheme in advance and are reminded to consider it during the laboratory practical. The staff who grade the reports have all lead the practical or have acted as teaching assistants and so have the experience to make necessary judgements. We do not make exemplars available to students, good or otherwise, as this would curtail thought and lead to slavish reproduction; this too is explained to students. We have designed earlier laboratory practicals to help students learn the key elements of effective experimental report writing (7) and so none of the features of the above scheme are unfamiliar to the students.

In summary, the report shows that it is possible to introduce of a level of inquiry into a first year laboratory practicals that are suitable for cohorts as large as 200.

ADDITIONAL RESOURCES

For additional information on this topic, any undergraduate level physiology or pharmacology textbook should provide relevant background information required to understand the theory this practical is based upon.

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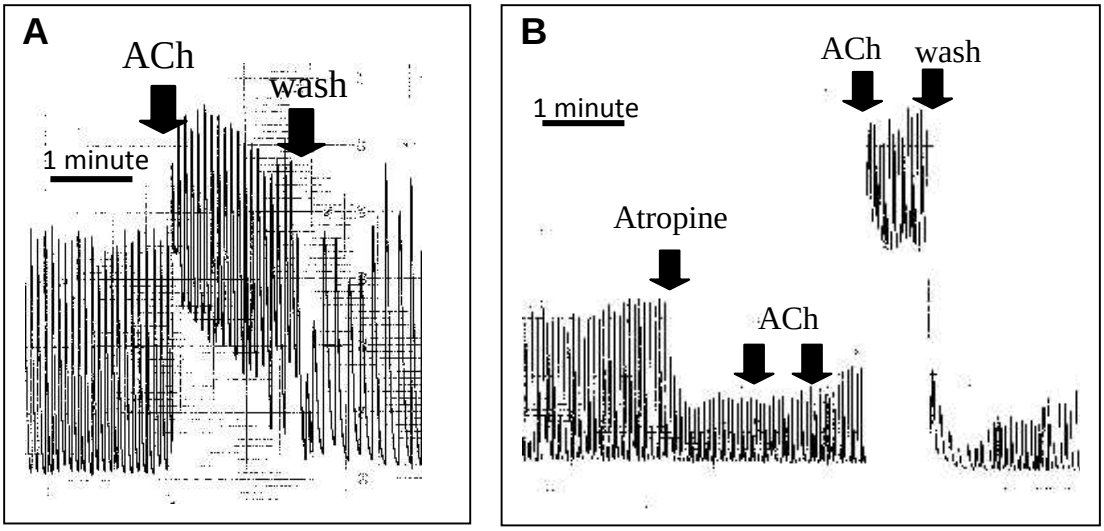

Figure 1. Example of two experiments that demonstrate the duration of drug exposure. **A**, acetylcholine alone, followed by wash (x2). **B**, Atropine, followed by acetylcholine (0.1 ml of solution 1), then a further 0.2 ml of solution 1 and finally 0.15ml of acetylcholine (solution 2). In ratio terms, these additions equate to 1x, 3x and ~10x. Part B demonstrates a characteristic property of competitive antagonism – the influence of concentration.

Drug name	Molecular weight (g)	composition of stock solution ($\mu g.ml^{-1}$)	Concentration (in molar terms) at tissue after adding 0.1ml of stock to the organ chamber that contains 20 ml. [Molar means moles per litre].
Acetylcholine 1	181.7	10	*$2.75x10^{-9}$ M (stock = $5.5x10^{-7}$ M)*
Acetylcholine 2		50	*$1.38x10^{-8}$ M (stock = $2.75x10^{-6}$ M)*
Noradrenaline 1	319.3	20	*$3.13x10^{-9}$ M (stock = $6.26x10^{-7}$ M)*
Noradrenaline 2		200	*$3.13x10^{-8}$ M (stock = $6.26x10^{-6}$ M)*
Adrenaline 1	219.7	20	*$4.55x10^{-9}$ M (stock = $9.1x10^{-7}$ M)*
Adrenaline 2		200	*$4.55x10^{-8}$ M (stock = $9.1x10^{-6}$ M)*
Phenylephrine	203.7	20.37	*$5x10^{-9}$ M (stock = $1x10^{-6}$ M)*
Isoprenaline	247.7	20	*$4.04x10^{-9}$ M (stock = $8.07x10^{-7}$ M)*
Atropine	694.8	20	*$1.44x10^{-9}$ M (stock = $2.88x10^{-7}$ M)*
Phentolamine	317.8	320	*$5.03x10^{-8}$ M (stock = $1.01x10^{-5}$ M)*
Propanolol	295.7	240	*$4.06x10^{-8}$ M (stock = $8.12x10^{-6}$ M)*

Table 1. Using the table above, calculate the molar concentration of the various drugs that will exist in the organ chamber assuming that 0.1 ml of the stock solution is added to 20 ml of physiological salt solution in the organ chamber. *Text in italic was missing from the student's version and the table's completion was a task for completion during the laboratory practical.*

Exp #	Drug(s) added	Receptor(s) target	Expected effect(s)	Observed effect(s)	Comments?
1	*Acetylcholine*	*mACh*	*Increase tone and/or amplitude of rhythmic contractions*	*Tone increased but amplitude of rhythmic contractions reduced*	*Force much increased as expected*
2	*Acetylcholine then atropine*	*mACh*	*Initially similar to exp #1 but then tone and amplitude reduced.*		
3					

Table 2. The truncated version of the '**table of likely effects**', as found in appendix 2, that teams are required to generate before starting to gather data. The table could be laid out prior to the laboratory practical by reference to the 'pre-practical support materials' (appendix 2), although additional rows will be required during the laboratory practical as additional experiments are designed to explore 'unexpected' observations.

Exp #	Drug(s) added	Receptor(s) target	Expected effect(s)
1	*Acetylcholine*	*mACh*	*Increase tone and/or amplitude of rhythmic contractions*
2	*Atropine*	*mACh*	*Variable but reduction in tone and amplitude commonly observed*
3	*Acetylcholine then atropine*	*mACh*	*Initially similar to experiment #1 but then tone and amplitude reduced towards control levels on application of atropine.*
4	*Noradrenaline*	*$alpha and beta*	*Decrease tone and/or amplitude of rhythmic contractions*
5	*Adrenaline*	*$alpha and beta*	*Decrease tone and/or amplitude of rhythmic contractions*
6	*Phenylephrine*	*alpha*	*Decrease tone and/or amplitude of rhythmic contractions*
7	*Isoprenaline*	*beta*	*Decrease tone and/or amplitude of rhythmic contractions*
8	*Phentolamine*	*alpha*	*Typically little or no effect*
9	*propanolol*	*beta*	*Typically little or no effect*
10	*Noradrenaline then phentolamine*	*$alpha and beta*	*Decrease tone and/or amplitude of rhythmic contractions but then tone and amplitude should increase towards control levels on application of phentolamine. Effect of phentolamine can vary – small responses should lead teams to increase concentration*
11	*Noradrenaline then propanolol*	*$alpha and beta*	*Decrease tone and/or amplitude of rhythmic contractions but then tone and amplitude should increase towards control levels on application of phentolamine. Effect of propanolol can vary – small responses should lead teams to increase concentration*
12	*Noradrenaline then phentolamine and propanolol*	*$alpha and beta*	*Decrease tone and/or amplitude of rhythmic contractions but then tone and amplitude should increase towards control levels on application of phentolamine and propanolol. Effect of antagonists can vary – small responses should lead teams to increase concentration of both antagonists*
13	*Adrenaline then phentolamine*	*$alpha and beta*	*Same as for noradrenaline but more variable*
14	*Adrenaline then propanolol*	*$alpha and beta*	*Same as for noradrenaline but more variable*
15	*Adrenaline then phentolamine and propanolol*	*$alpha and beta*	*Same as for noradrenaline but more variable*
16	*Phenylephrine then phentolamine*	*alpha*	*Initially the same as experiment #6 (decrease tone and/or amplitude of rhythmic contractions) but then tone and amplitude should increase towards control levels on application of phentolamine.*

17	*Phenylephrine then propanolol*	*$alpha and beta*	*Should be the same as experiment #6 as propranolol (beta-selective) should NOT antagonise phenylephrine (alpha agonist)*
18	*Isoprenaline then propanolol*	*beta*	*Initially the same as experiment #7 (decrease tone and/or amplitude of rhythmic contractions) but then tone and amplitude should increase towards control levels on application of propanolol*
19	*Isoprenaline then phentolamine*	*$alpha and beta*	*Should be the same as experiment #7 as phentolamine (alpha-selective) should NOT antagonise isoprenaline (beta agonist)*
20	*Acetylcholine then noradrenaline*	*[$]alpha, beta and mACh*	*Similar outcome to experiment #1 (increase in tone and amplitude of rhythmic contractions) but then tone and amplitude are reduced towards control levels on application of noradrenaline. The effect of noradrenaline should be negated by prior application of both phentolamine and propanolol*
21	*Acetylcholine then adrenaline*	*[$]alpha, beta and mACh*	*Same as experiment #20*
22	*Noradrenaline then acetylcholine*	*[$]alpha, beta and mACh*	*Similar outcome to experiment #12 (decrease tone and/or amplitude of rhythmic contractions) but then tone and amplitude increased towards control levels on application of acetylcholine. Effect of acetylcholine can vary – small responses should lead teams to increase concentration of acetylcholine. Effect of acetylcholine could be negated by prior application of atropine*
23	*Adrenaline then acetylcholine*	*[$]alpha, beta and mACh*	*Same as experiment #22*

Table 3. **Complete table of effects**. Students are not provided with the complete table but are expected to generate it before and during the laboratory practical using information in the 'pre-practical support materials' (appendix 2), their textbooks and lecture material. Experiments 20 to 23 (shaded) would be appropriate if students were encouraged to demonstrate for themselves the principle of physiological antagonism.

[$]No order of potency is given and this is deliberate. Students are often frustrated by seeming contradictions when researching the question of potency. This is likely explained by the use in historical research of different preparations from different species in which the specific receptor subtypes (alpha 1 and 2 and beta 1 and 2) differ or else their relative densities vary. Suggesting that both agonists act on both alpha and beta receptors appears to be accepted by students and their minds remain open to observation.